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REVIEW

A history of research into the physiology of bile, from Hippocrates to molecular medicine

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INTRODUCTION

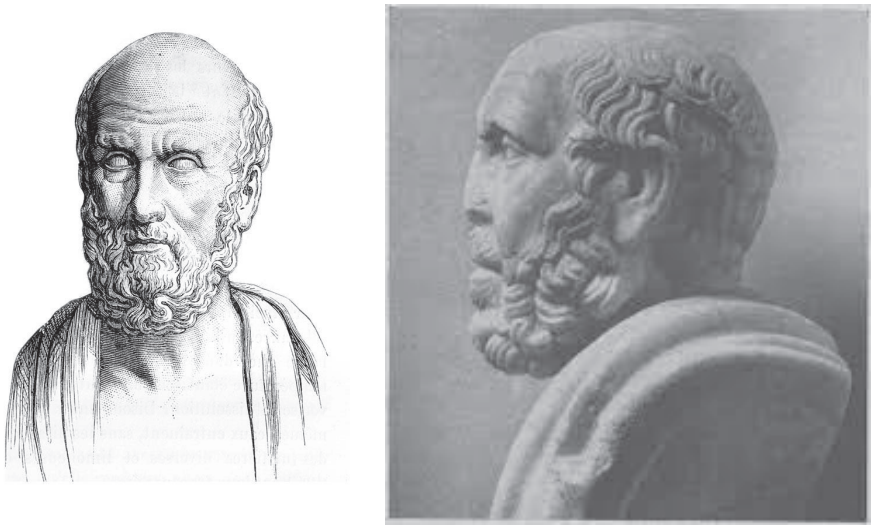
The earliest known mention of bile, for use in enemas and other treatments, is found in Egypt in the Ebers Papyrus of the 16th century BCE.[1] Yet references to the liver and bile, respectively, were far from isolated in the ancient world, understandably so considering the impressive sight of the largest internal organ in the body filled with bright red blood—the life force—as depicted in the Upper Paleolithic wall painting of an eviscerated bison in the caves at Lascaux (near Montignac in the Dordogne region of southwestern France) and the unique body fluid with its distinctive yellow-green color, odor, and bitter taste, which dictated its etymological designation and metaphors. In old Hebrew, the label used *poison* (ראש; see Deuteronomy 32:33[2] and Matthew 27:34[3])[2–4] focused on the taste of bile, whereas the Proto-Indo-European epithet *ǵʰelh₃- ("green, yellow") dwells on its color and gives cognates in Old Latin *fel*, *holus*, *helvus*; in Ancient Greek *choli* χολή and *chloros* χλωρός that meant "yellow" and metaphorically "anger and indignation" in Latin (*cholera* from the Greek); in English *gold* and Old English *ġeolu* ("yellow"); and a slew of other languages.* The alternative term in Latin, *bilis*,† also denotes a yellow *bitter* liquid secreted by the liver that aids in digestion.[5] The Latin appellation *cholera* that was used for bile until the 18th century could, like *bilis*, also signify a touchy irascible disposition because of an excess of black bile (see later); occasionally bile and liver were metaphors for courage, anger, arrogance, daring, and amorous tendencies,‡ even lust,[6] in English[7] and other languages.[8] Biliousness was a common symptom in Britain in the 18th century, probably related to being *liverish* over the next 100 to 200 years or so.[9,10] Yet liverish was considered a functional rather than an organic disorder of the liver, even though the general malaise and dyspepsia and indeed bad temper that the term liverish suggested had actually been recognized by army surgeons at the time of the British Raj as premonitory warnings of a liver abscess.[9] The French, of course, have their *crise de foie* and *colique hépatique*, but when a Frenchman was bad tempered, he was said to be *un bilieux*.

It was therefore logical to the physician of old that bile should play an important role in physical health, as well as in mood and temperament—that is, a "proportioned mixture of elements," from Latin *temperare* to control or blend together the four qualities: hot, cold, moist, and dry. A mainstay of the disciples or school of Hippocrates (c.460–c.377 BCE; Figure 1) was the humoral theory of diseases, a concept that originated with the Pythagoreans two centuries earlier. Health and disease were thought to be dependent on the balance between the four main humors or body fluids, that is, the *choleric* warm dry yellow bile, the *melancholic* cold dry black bile, the *sanguine* warm moist blood, and the *phlegmatic* cold moist phlegm (Figure 2A), corresponding, respectively, to the four qualities and the four basic elements, namely, fire, earth, air, and water (Figure 2B). The Hippocratic composition that describes in detail the formation of bile and the way bile increases in the body is in *Diseases IV*.[11] In contrast with earlier work in which the Greek physician distinguished blood, phlegm, yellow bile, and black bile, in *Diseases IV*, Hippocrates mentions blood,

*https://en.wiktionary.org/wiki/Reconstruction:Proto-Indo-European/ǵʰelh₃-, last accessed December 30, 2021.

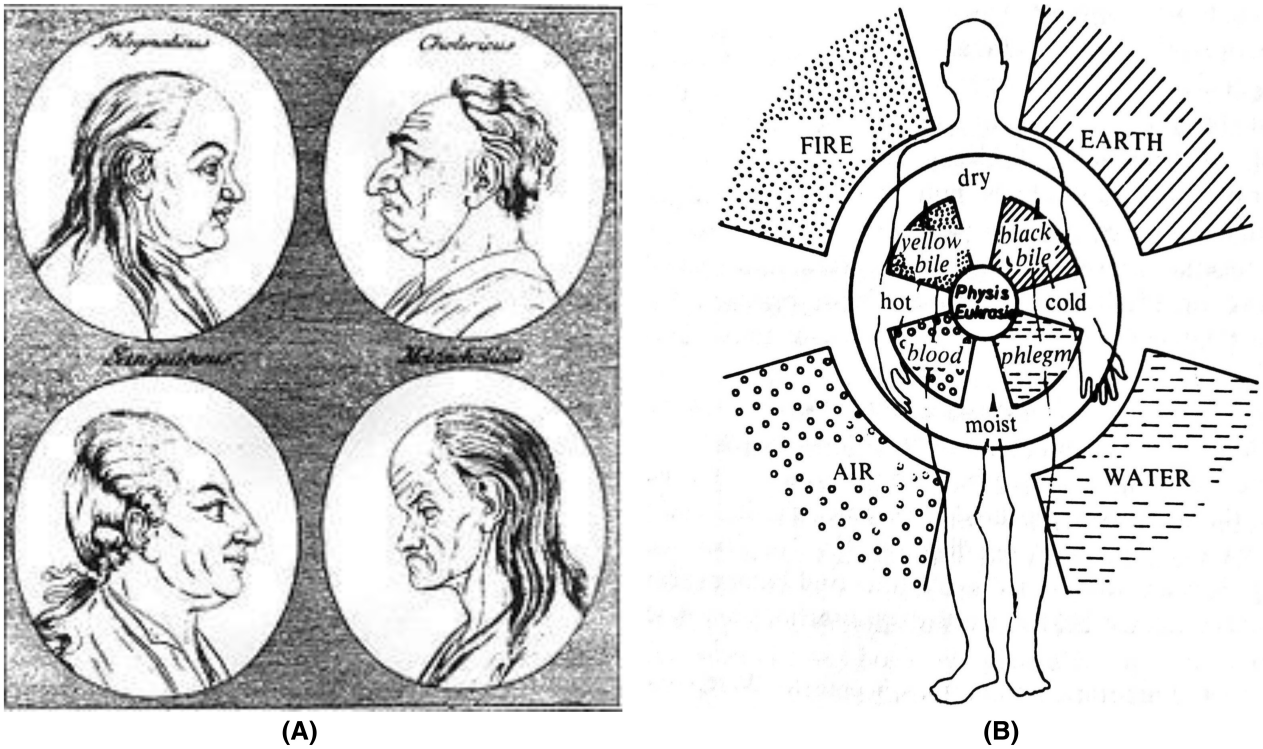
†https://www.wordsense.eu/bilis/, last accessed December 30, 2021.

‡https://www.etymonline.com/word/bile, last accessed December 30, 2021.



(A) (B)

FIGURE 1 The Greek physician Hippocrates of Cos (c.460–c.377 BCE) was the first to propose that the "source of bile was part of the liver." (A) Line drawing portrayal of Hippocrates; this drawing was copied from the first edition of the works of Hippocrates by Francisco Asulanus, edited by the Publishing House of Aldo Manuzio (Aldus Manutius), Venice in 1526. (B) Side view of a sculptured head of an elderly man, which was discovered in 1940 lying on the ground in Antica in Northern Ostia at the end of the Via Flavia, in front of the tomb of K. Markios Demetrios, a distinguished Greco-Roman physician of the 1st century CE. The bust, which was a Roman copy made of Parian—Greek white marble—of an earlier 3rd century BCE Greek sculpture, was identified as Hippocrates by Prof. Giovanni Becatti (1912–1973), an Italian Classical Art Historian and Archaeologist of the University of Pisa, who directed excavations at Ostia Antica, as reported by D.W. Richards.[12] (A) Reproduced with permission from *British Medical Journal*.[9] Copyright 1938, British Medical Association.



(A) (B)

FIGURE 2 (A) Image of woodcut from Physiognomische Fragmente, zur Beförderung der Menschenkenntnis und Menschenliebe (1775–1778) by Johann Caspar Lavater, depicting the four humors: phlegmatic (upper left; i.e., mucus phlegm, phlegma φλέγμα), choleric (upper right, i.e., yellow bile, xanthe chole ξανθη χολή), sanguine (lower left, i.e., blood, haima αἷμα), and melancholic (lower right, i.e., black bile, melaina chole μέλαινα χολή). (B) The association between the four elements—fire, earth, water, and air (depicted on the outside of the figure)—and the four Hippocratic humors (yellow bile, black bile, blood, and phlegm), enveloped in the inside by the four qualities: dry, moist, hot, and cold. The natural state (physis, φύσις), that is, good health (eucrasis/eucrasia, εὐκρᾶσις/ία) is achieved when there is a proper blending and balance of the elements, qualities, and humors in amount and strength. When these are disproportionate, disease dyscrasia (δυσκρασία) ensues. Reproduced with permission from *History of Physiology*.[96] Copyright 1973, RE Karger Publishers Co.

FIGURE 3 Eighteenth-century engraving of Claudius Galenus by the Prussian engraver Georg P. Busch (1707–1756) (Berlin). Reproduced with permission from The Wellcome Collection, London.

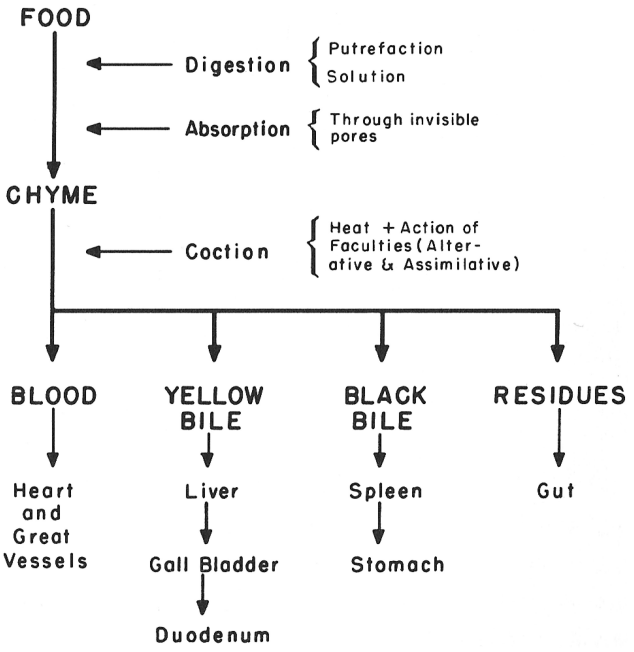


FIGURE 4 Physiology of digestion and sanguification (manufacture of blood) according to Galen. Chyle from food partially digested in the stomach and to some extent from the small intestine passes in the portal vein to the liver where the process of digestion (*coction*) is completed. Partially concocted food material (chyme) also passes from the stomach into the gastrointestinal tract. Excrement from concoction in the liver is excreted as yellow bile into the intestine by way of the gallbladder and bile duct, and as black bile after passage in a retrograde manner through the portal vein, splenic vein, and spleen, by way of a hypothetical splenic duct into the stomach. The heavy earthy material that remains within the gut after digestion is excreted as feces mixed with black and yellow bile. Reproduced with permission from figure 11 in *Circulation of the Blood: Men and Ideas*.[14] Copyright 1964, Oxford University Press.

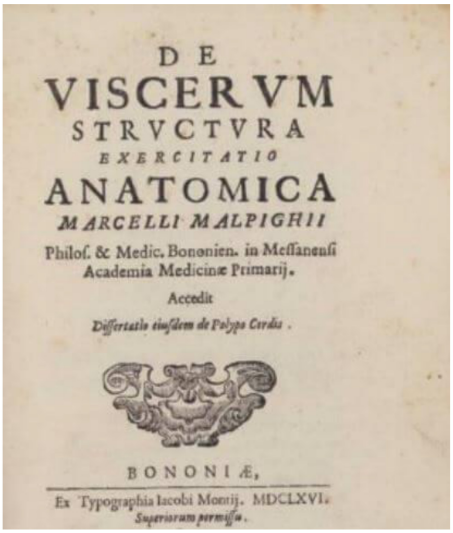
phlegm, bile, and *water*. The balance (*physis* φύσις) between these four humors, an extension of the *harmony of opposites*, guarantees good health (Figure 2B). Hippocrates clearly writes that the "source of bile is the part of the liver" (probably the gallbladder).[12,13]

Later, Aelius/Claudius Galenus (Κλαύδιος Γαληνός), anglicized as Galen of Pergamon (129–c.216 CE) (Figure 3), one-time physician to the gladiators of the Temple of Pergamon's High Priest and physician to several emperors in Rome, also proposed that yellow bile comes from the liver. It was not that Galen was concerned especially with bile formation per se. Rather, he attempted to unify those ancient notions of human physiology—albeit only those *with which he agreed*—dating from the Babylonians, the Egyptians, the Etruscans, and especially the Greeks, who were particularly preoccupied with the location and nature of the soul, as well as the mechanisms responsible for health and disease. Galen fancied a dominant role for the liver as the "seat of sanguification—that is, the manufacture of blood[§]—and the source of the veins." In his schema for the process of digestion (Figure 4),[14] Galen incorporated the concept that the stomach could separate the useful from the useless constituents of food as chyle and provide for the former an absorption site into the portal vein for transport to the liver. Using *innate heat* or the heat of vegetative *pneuma* (i.e., the spirit) derived from inspired breath, the baser nutritive liver component of the tripartite soul (in contrast with the rational and noble, and the spirited, emotional, and affective thirds of the soul that are located in the brain and heart, respectively) completes the *concoction* by which blood is elaborated from chyle. More than a millennium later, Jean François Fernel (Latinized as Ioannes Fernelius), the eminent 16th-century French physician (1497–1558), mathematician, and astronomer,[¶] who coined the neologism *Physiology*, concurred with this *second*

[§]To which idea the rabbis of the Talmudic period (70–640 CE) and *even* William Harvey (1578–1657) subscribed.

[¶]After whom the Fernelius lunar crater is named.

(A)



(B)

FIGURE 5 (A) Late 17th-century line engraving of Marcello Malpighi by Jan Kip, after an unknown artist. Source: National Portrait Gallery, London. (B) Front cover of a rare first edition of Malpighi's De Viscerum Structura Exercitatio Anatomica, published by Giacomo Monti in Bologna in 1666, from the collection of Irwin J. Pinkus, MD. Auction at Christie's closed on December 6, 2004.

concoction. Incidentally, chyle was also cleansed of impure residues that become fecal matter. Galen thought that blood made by the liver could contain phlegm, air, and black bile,[15] but he opposed the view of the distinguished Alexandrian anatomist and physiologist Erasistratus of Chios (310–250 BCE) that arteries contained air.[16] Incidentally, Galen mocked Erasistratus's prescient hypothesis that tiny channels exist in the liver, connecting the portal to the hepatic veins.

However, the proof that bile is really produced by the liver had to wait until the 17th century and the work of Marcello Malpighi (1628–1694)[17] (Figure 5), who is considered to be the founder of microscopical anatomy and histology, the father of physiology and embryology, and arguably the patriarch of plant anatomy as well.[18] Even though Malpighi has some 50 structures named after him, at least in Italian anatomy, arguably his greatest discovery was that of the hair-like vascular structures in the frog's lung,[19] for which he coined the term *capillaries*, after *capillari*—from the Latin *capillus/capillum* for hair. Malpighi first communicated his discovery of the structure of the lung in two letters to his long-time friend from his days in Pisa,[20] Giovanni Borelli, but later published it formally after an invitation to present his work at The Royal Society in London.[21] Notwithstanding, *biliophiles* should value highly Malpighi's appreciation of the acinar organization of the liver** and his recognition that bile originated from the liver lobule and not the gallbladder,[17] as was popularly imagined and remained so until the mid-18th century. Malpighi's decisive experiment-based view of hepatic bile formation was not without its deluded antagonists but was soon

**Resurrected later by Aron Rappaport.[23]

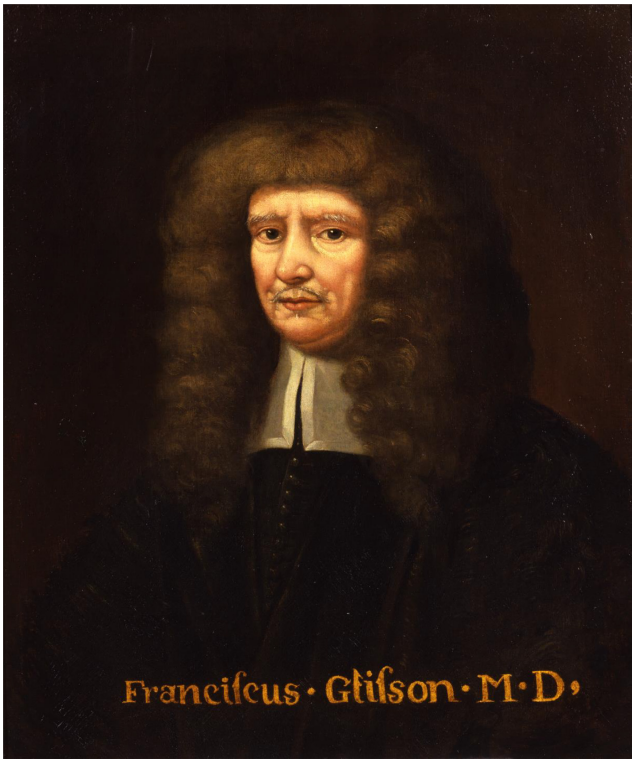


FIGURE 6 Portrait of Francis Glisson located in the Museum of the Royal College of Physicians London. © Royal College of Physicians. It is arguable that this portrait was painted by William Faithorne (c.1620–c.1691), who was the artist responsible for the line drawing engraving that is found in the National Portrait Gallery and the Wellcome Institute, because Faithorne was not accustomed to painting in oils.

echoed enthusiastically by Francis Glisson[23] (1598/9–1677; Figure 6) in London. Glisson envisioned bile secretion as a process of filtration from the blood, although

FIGURE 7 (A) Portrait of William Harvey in the Museum of the Royal College of Physicians London. © Royal College of Physicians. This likeness that was painted around 1650 shortly before Harvey's death has been attributed to the Dutch artist Cornelius Johnson, but this is disputed. This portrait was one of only two paintings rescued from the Royal College of Physicians during the great fire of London in 1666; the heavy restoration that was necessary is evident in Harvey's distorted right hand. (B) Terracotta statue of William Harvey. Charles Bell Birch (1832–1893) and the Torquay Terracotta Company (1886). Reproduced by kind permission of The Royal Society of Medicine, London, where the statue is located in the Heritage Centre on the second floor of the library.

he could not visualize the necessary connections between the vascular and biliary systems. Yet despite this insight, Glisson actually proposed that the flow of bile results from the successful effort by the ducts to expel the *irritating* contents. Glisson further theorized that "irritability" was a vital property of all tissues, but this once-popular doctrine of physiology was abandoned in the 18th century.[24] Although not necessarily an adherent to the Irritability Doctrine of his close contemporary, William Harvey (Figure 7) endorsed Glisson's vision of the excretory/secretory nature of bile formation[††] and foresaw its detergent and cathartic properties in the intestine.[25] Harvey's intuition about the digestive role of bile in intestinal function was richly reciprocated over the succeeding centuries in studies on digestion by a luminary roster of *lumenary* 16th- to 19th-century physiologists.[‡‡] But we digress.

Criticisms by Glisson's detractors verged on mockery,[27] but their scepticism was bolstered by Glisson's own inconvenient observation that blood flow in the portal system was slow and nonpulsatile. The inescapable objection to blood flow–based bile formation was resolved when an alternative explanation to a hydrostatic mechanism for bile flow was demonstrated,[28] and the intricacies of portal hemodynamics were fully elucidated as the mysteries of portal hypertension were revealed.[29] Yet by the end of the 18th century, the impression that bile formation was the only recognized function of an organ as large as the liver was met with incredulity by the anatomic physiologists of the day.[30]

It was also during the 17th century that the concept of an enterohepatic cycle emerged, which during the 18th century was seen as a mechanism to conserve the constituents of bile. The term "bile acids" was proposed by von Liebig in 1842,[31] and it was only in 1870 that the remarkable physiologist Moritz Schiff unequivocally described the enterohepatic circulation.[32] The cellular and molecular mechanisms that underlie this cycle were discovered much later. Surely the history of bile acids deserves its own essay in the current series? Meanwhile the reader is directed to the tour de force four-score-year history of key discoveries in bile acid chemistry and biology authored by Hofmann and Hagey[33] and the equally masterful review of cholesterol gallstone pathogenesis by Frank Lammert[34] that will appear soon in the current series.

[††]From his years of tenure as the renowned Lumleian lecturer,[27] which began in April 1616. Harvey's manuscript notes of these lectures "Praelectiones anatomicae Universal For me Gulielmum Harveium, medicum Londinensem, anatomist. and surgeon. Professor, Year Sun. 1616, aetatis 37: praelect. April, 1617," were rare documents that were rescued from the Great Fire, which engulfed the library that Harvey helped establish at the Royal College of Physicians, and are now in the British Museum.

[‡‡]Sixteenth century: Jean François Fernel and Philip Aureolus Theophrastus Bombastus von Hohenheim (i.e. Paracelsus); 17th century: Jan Baptist van Helmont and Isbrand van Diemerbroeck; 18th century: Herman Boerhaave and Albrecht von Haller; 19th century: Theodore Schwann, Reinhold Schellbach, Johannes Peter Müller, William Saunders, and William Beaumont.

The mechanisms by which the liver produces bile were progressively elucidated during the second half of the 20th century. Ralph W. Brauer and colleagues,[28] at the US Naval Radiological Defence Laboratory in San Francisco, California,[§§] were the first to address the question of the source of energy for bile formation. At that time, it was well established that the energy for urine secretion was hydrostatic pressure provided by the heart contractions and transmitted to renal arteries and capillaries. To test the hydrostatic pressure hypothesis for bile secretion, Brauer used the isolated perfused rat liver with a cannula inserted into the common bile duct and placed in a vertical position, to gauge bile secretory pressure. He could monitor the arterial perfusion pressure simultaneously and, perhaps to his surprise, he saw that the bile column always rose to a higher level than the arterial perfusion pressure, whatever latter pressure he used (above a threshold). This showed conclusively that hydrostatic pressure could not be the source of energy for secretion of bile by the liver. Brauer and colleagues[28] concluded that bile formation required metabolic energy and a process of "active transport." The hypothesis at this stage was that the initial process of bile formation was not hydrostatic filtration but rather osmotic filtration in response to the active transport of one or several solutes into the canalicular lumen.

Five years later, in a landmark paper, Ivar Sperber,[35] from Uppsala (Sweden), proposed that "the primary event in bile formation would be the active transfer (from the cells or through the cells) of bile acids (and possibly other, less quantitatively important compounds) into the bile *capillaries* that nowadays are called bile canaliculi.[22] The osmotic effect of these solutes would result in a flow of water and dissolved molecules and ions into bile capillaries. In support of this theory, Sperber[35] showed experimentally that bile flow was positively related to bile salt excretion rates in bile. His theory was directly derived from his observations on renal physiology and the relationship between organic anion secretion and the flow of urine.

The hypothesis proposed by Sperber[35] was strongly supported a few years later by Henry Wheeler and his coworkers at Columbia University in the laboratory of Stanley Bradley. Wheeler et al.[36] used inulin and mannitol as markers of canalicular bile formation in bile fistula dogs. They proposed that mannitol enters canalicular bile by diffusion and is neither reabsorbed nor secreted by the biliary channels, so that its secretion rate is an estimate of canalicular bile flow. They showed that when sodium taurocholate was infused at increasing rates, both bile flow and mannitol excretion increased, but when the hormone secretin was given, bile flow increased but mannitol excretion remained stable.[36] The observation that secretin does not increase mannitol excretion is consistent with the idea that secretin stimulates secretion by the biliary epithelium (in the same way that it stimulates secretion by pancreatic ducts). These observations in four dogs were similar to the findings in guinea pigs by Lee Forker[37] and earlier by Chenderovitch et al.[38]

The interpretation of these observations was complicated by the fact that mannitol is a marker of extracellular space and as such, it should not enter the hepatocyte. This apparent contradiction was resolved with the discovery of aquaporins.[39] Aquaporin 9 (also known as aquaglyceroporin, because it allows the movement of glycerol) is found on the basolateral membrane of hepatocytes[40] and allows the transport of mannitol into the hepatocyte. Because aquaporin 9 is not found on the canalicular membrane, it is likely that mannitol enters canalicular bile by a vesicular mechanism related to bile acids.

At this stage, it was reasonably well established that there are two sites of bile formation: the canaliculus and the bile ductules or ducts. Canalicular bile flow is stimulated by bile acids, while ductular/ductal bile flow is stimulated by secretin. Water movement in the bile ducts or ductules occurs through aquaporin 1,[41] which does not transport mannitol. This explains why secretin does not increase mannitol clearance.

In their original description of the linear relationship between canalicular bile flow and bile salt secretion, Wheeler et al.[36] noted that this relationship, when "extrapolated" to zero bile salt secretion, yielded a positive value. They postulated that this corresponded to a bile salt–independent fraction of canalicular bile flow. With Micheline Dumont and others in our laboratory,[42–44] we showed that this component of canalicular bile flow was much greater in rabbits and rats than in the dog. Jim Boyer[45] at Yale University confirmed this independently in the isolated perfused rat liver. This concept was challenged because it was argued that the "osmotic activity" of bile salts increases at low bile salt concentrations,[46,47] and hence at low bile salt concentrations and excretion rates, a given amount of bile salts could drive more water than at higher concentrations. However, several other observations support the view of a canalicular bile salt–independent flow in all species studied[48–50] (Figure 8), including humans.[51,52] We initially proposed that canalicular bile acid–independent bile flow was driven by Na^+ transport into canaliculi mediated by the enzyme Na^+, K^+-ATPase.[42] This hypothesis was refuted when it was shown that Na^+, K^+-ATPase was not located on the canalicular membrane but on the basolateral membrane of hepatocytes. There is now evidence that canalicular bile salt–independent flow is driven by secretion into the canaliculi of glutathione and other thiols[53,54] and bicarbonate.[55,56] Bicarbonate secretion by the hepatocyte is mediated by a canalicular Cl^-/HCO_3^- exchanger.[57,58]

[§§]This was an early military laboratory created in 1946 to study the effects of radiation and nuclear weapons. The facility was based at the Hunter's Point Naval Shipyard in San Francisco, California, to manage testing, decontamination, and disposition of US Navy ships contaminated by the pair of Operation Crossroads nuclear tests at the Bikini Atoll in the Pacific.

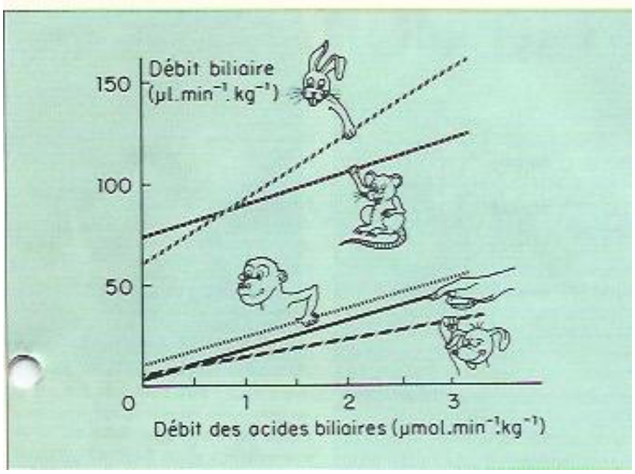


FIGURE 8 Relationship between bile flow and bile acid secretion rate in five species (rabbit, rat, dog, monkey, and human). The relation extrapolates to a positive value for a zero bile salt secretion, an estimate of the bile acid–independent bile flow. *Débit biliaire* = bile flow; *débit des acides biliaires* = bile acid secretion rate. Reproduced with permission from *La Revue du praticien*.[52] Copyright 1991, J.-B. Baillière.

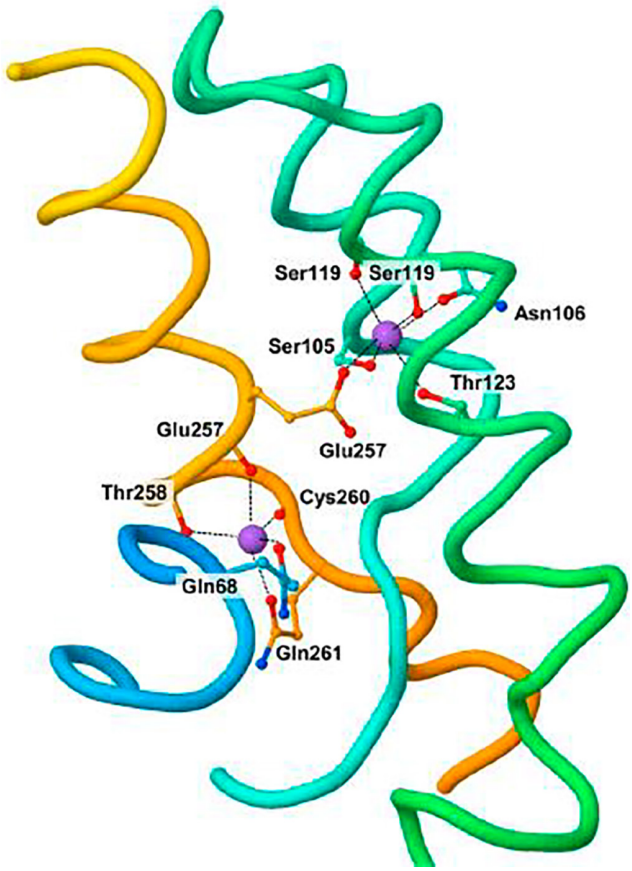


FIGURE 9 Three-dimensional structure of sodium taurocholate cotransporting polypeptide (SLC10A1), the sodium taurocholate basolateral transporter of hepatocytes. Reproduced with permission from *Current Topics in Membranes*.[72] Copyright 2012, Elsevier.

The next major step in understanding the mechanisms of bile secretion was to identify the membrane and cellular transporters mediating these different functions. The protein responsible for bile acid transport across the canalicular membrane was identified by photoaffinity labeling in 1991 by Müller in the Dietrich Keppler laboratory[59] and by Nishida working with Win Arias.[60] Then, Emmanuel Jacquemin, working in the late Peter Meier's laboratory in Zurich, made an important advance. He cloned the first organic anion transporter, called organic anion transporting polypeptide (OATP),[61,62] located on the basolateral membrane of hepatocytes and responsible for the hepatocellular uptake of the dye sulfobromophthalein (bromsulfthalein), which was once used extensively in humans (both clinically and experimentally) and animals in testing liver function[63] and of the sodium-independent uptake of conjugated bile acids. Since then the group of OATPs (now named solute carriers [SLCs]) has been considerably extended: it includes more than 300 members organized into more than 60 families.[64] Most members of the SLC group are located in the cell membrane. The molecular cloning of OATP (SLC) was followed by that of the sodium taurocholate cotransporting polypeptide (the sodium-dependent transporter of bile acids, also known as SLC10A1), which is expressed on the basolateral membrane, by Bruno Hagenbuch[65] in the same laboratory in Zurich. This pioneering work opened a new chapter in the understanding of biliary physiology, namely, the molecular cloning of the membrane transporters responsible for the secretion of all biliary constituents. The list of these transporters is quite long and has been the subject of several reviews,[66,67] regarding both hepatocytes and cholangiocytes.[68,69] Soon the three-dimensional structure of these transporters was established, along with identification of mutant forms.[70] An example is given in Figure 9. The identification of the transporters implicated in bile formation led to an understanding of several genetic diseases caused by mutations of the carrier proteins,[71] mirroring remarkable progress in the knowledge of the mechanisms of hereditary hyperbilirubinemia and cholestatic syndromes.[72]

As for the mechanism for bile, *once formed*, to flow downstream *out of* the canaliculi, it stands to reason that it must be hydrostatic because the primary force for secretion by hepatocytes is the osmotic *pressure* generated by concentrative bile acid translocation across the canalicular membranes. In the absence of bile secretory failure or biliary obstruction, bile flows from the canaliculi via the canals of Hering[22] into the biliary tree. Although not subscribing to Glisson's irritability hypothesis, bile flow from central regions of the lobule to the bile ducts is enhanced by canalicular “peristalsis” because of coordinated calcium-stimulated contractions of pericanicular actin-myosin microfilaments.[73,74]

Perhaps more surprising than the finding of canalicular peristalsis upward of 40 years ago comes the reborn hypothesis that hydrostatic pressure (because of paracellular water movement) may indeed play a role in bile formation in the human liver. This and other developments in the field have recently been comprehensively reviewed.[67]

Another major step in our knowledge of biliary physiology was the discovery in 1999 that bile acids serve as ligands for the nuclear receptor FXR (the farnesoid X receptor).[76–78] This opened the way to characterize their actions as selective signaling molecules[79] for, as we shall see, their therapeutic potential.

Medical research has a major goal, which is to improve health. Research on biliary physiology has fully reached this goal. The first medical application of bile acids in medicine was found in Asia. Chinese traditional physicians have used bear bile to treat various digestive disorders for centuries[79] a tradition that was extended to Japan and Korea. Bear bile contains ursodeoxycholic acid—from *Ursa*, the Latin for bear—the 7β epimer of chenodeoxycholic acid from *chena*, χήνα, Greek for goose. In 1972, Alan Hofmann and colleagues were the first to demonstrate that chenodeoxycholic acid was able to dissolve radiolucent (cholesterol) gallbladder gallstones,[80] the first medical treatment of this disease. However, chenodeoxycholic acid had a few side effects, including diarrhea and elevations of aminotransferases, and was superseded by ursodeoxycholic acid, after Makino et al.,[81] in 1975, showed that cholesterol gallstone dissolution could be obtained, with very few side effects. This initiated a considerable number of clinical studies all over the world.[82] The multiple physiological, pathophysiological and therapeutic aspects of bile acids were discussed with great enthusiasm at the regular bile acid meetings organized generously by Herbert Falk (1924–2008) alternately in Freiburg and Basel. These scientifically and socially lavish meetings undoubtedly contributed to progress in the discipline.

Ursodeoxycholic acid was largely used in selected patients with gallstones until the advent of laparoscopic cholecystectomy in the early to mid-1980s, chronologically in Russia, Germany, and France. For a while, the invention and innovation of laparoscopic cholecystectomy were enigmatic,[83] and its pioneers were not immediately recognized or applauded. Philippe Mouret, a French surgeon in Lyon, did not submit his work for publication because he "did not see any chance for publishing in a surgical journal," as he stated in 1994 in an interview with Litynski.[84] But, with the help of his colleagues François Dubois in Paris and Jacques Périssat in Bordeaux, laparoscopic cholecystectomy rapidly gained widespread acceptance among surgeons and the public. It also developed very rapidly in other European countries and in the United States. In a similar hostile environment, Erich Mühe (1938–2005), an Erlangen-trained surgeon

FIGURE 10 Erich Mühe, innovative surgeon and 1985 national and 1987 international cycling champion. Reproduced with permission from *Journal of Minimal Access Surgery*.[83] Copyright 2011, Indian Association of Gastrointestinal Endo Surgeons.

(Figure 10) working in Böblingen, Germany, performed laparoscopic cholecystectomies a few years before Mouret, but Mühe's work was rejected by the German surgical community and remained largely ignored.[85] The colorful, often controversial history of gallstone surgery and its eventual acceptance is told with panache by Frank Lammert[34] in another forthcoming essay in this series, in which the pathogenesis of cholelithiasis is also described, lyrically. Since the advent of laparoscopic cholecystectomy, the medical dissolution of gallstones by ursodeoxycholic acid rapidly lost its indications and the favor of physicians.

However, ursodeoxycholic acid rapidly knew an exciting rebirth. With Raoul Poupon, working with me, we reasoned that ursodeoxycholic acid was a "very special bile acid" with properties quite different from those of physiological bile acids. We had discovered, with Micheline Dumont, that it was "hypercholeretic," with a marked increase in bicarbonate output into bile[86] (Figure 11). In addition, in contrast with physiological bile acids, it was hydrophilic, not detergent, and was not toxic to cell membranes. Because it was already approved as a drug by health agencies, Poupon[87] decided to try it in patients with primary biliary cholangitis (PBC) (termed primary biliary cirrhosis at that time). The first results were spectacular in terms of serum liver biochemical tests,[87] and a controlled trial showed a highly significant prolongation of transplant-free survival in treated patients.[88] This result was confirmed by a combined analysis of several international trials,[89] and now

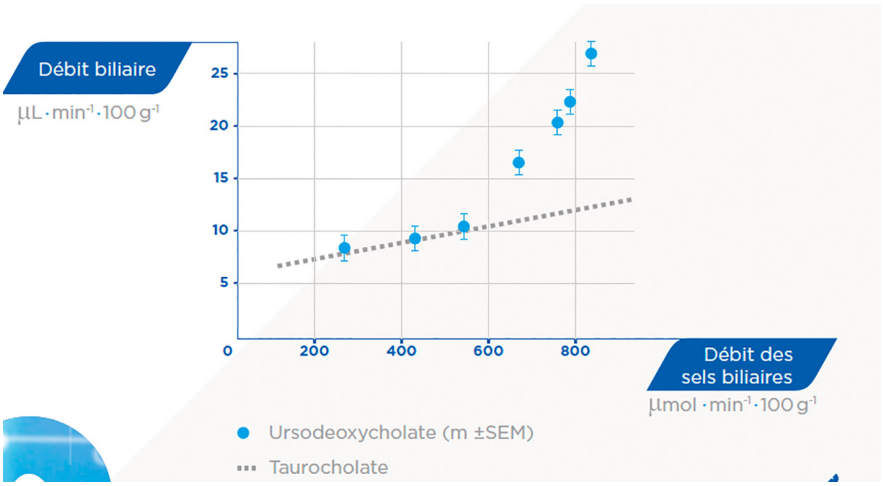


FIGURE 11 Hypercholeresis induced by ursodeoxycholate in the rat. At secretion rates greater than 500 µmol/min/100 g of body weight, ursodeoxycholate has a much greater choleretic effect than taurocholate. *Débit biliaire* = bile flow; *débit des sels biliaires* = bile salt secretion rate. Reproduced with permission from *Gastroenterology*.[86] Copyright 1980, American Gastroenterological Association.

ursodeoxycholic acid is universally recommended as a first-line treatment of PBC[90] and of a number of cholestatic diseases.

The discovery that bile acids are ligands of the nuclear receptor FXR also opened a new therapeutic avenue. When they bind to FXR, physiological bile acids repress the synthesis of new bile acids through inhibition of 7α-hydroxylase, a rate-limiting enzyme in bile acids synthesis.[9] This is a "protective" mechanism against the accumulation of bile acids in liver cells during cholestasis. Obeticholic acid, or 6-ethylchenodeoxycholic acid, is a much more potent agonist of FXR than physiological bile acids. When administered to patients with cholestasis, it blocks efficiently the synthesis of new bile acids, decreases bile acids in the liver, and improves markers of cholestasis. It has been successful in decreasing alkaline phosphatase and other biochemical tests in patients with PBC[91,92] and, more surprisingly, in patients with nonalcoholic fatty liver disease (NAFLD[¶¶]). The interim results of an ongoing multicenter randomized trial[94] in this latter disease have been reported,[95] in which obeticholic acid 25 mg significantly improved fibrosis and key components of disease activity among patients with nonalcoholic steatohepatitis, and this is reasonably likely to predict clinical benefit.

Physiological advances in the knowledge of bile formation led to therapeutic advances that have already helped many patients and, it is hoped, will continue to do so. The history of research on bilirubin, the pigment responsible for jaundice, is also a fascinating area and will be the subject of another essay in this History of Hepatology series by Toni Herta and Ulrich Beuers.

[¶¶]Recently suggested to be renamed metabolic-associated fatty liver disease (MAFLD[94]).

SERIES EDITOR'S POSTSCRIPT

The gradual development of our understanding of how bile is formed exemplifies the changes in the study of the liver that have taken place over the millennia, and especially during my own investigative professional career that began in earnest enquiring into biliary lipid secretion in obese individuals in late 1976. Given the profusion of functions of the liver, the investigation of which is ably reviewed by Mousa and Kamath[64] in the current series, it is curious to reflect that at the end of the 18th century bile secretion was deemed the sole justification for hepatic existence, to which the liver was relegated once the soul was relocated elsewhere. In this context, there is no one better than Serge Erlinger to relate the "History of Research Into the Physiology of Bile," because he was a key contributor to our understanding of bile formation, for which he was duly recognized and honored by the scientific hepatology community, including being awarded the coveted quadrennial prize by the Falk Foundation in 1980.

As with other developments and discoveries in the history of hepatology, serendipity can be relied on to play its role here too, as long as *prepared minds are on hand to be favored by chance*.[***] How else can one explain that a dihydroxy-5beta-cholanic acid derivative of chenodeoxycholic acid, obeticholic acid, a FXR agonist that is a useful adjunct in the treatment of the cholestatic liver disease PBC, also shows promise in the treatment of NAFLD.

Prof. Erlinger is a native *Parisien*, whose medical studies were at the University of Paris (1957–1963), his residency (1962–1967) was fulfilled at Paris hospitals, and a research fellowship was undertaken at New York-Presbyterian/Columbia Hospital in Stanley E. Bradley's

[***]According to Pasteur's famous aphorism.

laboratory. Under the direction of Prof. René Fauvert, he was awarded his Doctorate in Medicine in 1967 for his thesis on the mechanisms of bile secretion—what else? His glowing career thereafter included time (1973–1976) at the Institut National de la Santé et de la Recherche Médicale (INSERM) and a rise within the ranks at l'Hôpital Beaujon in Clichy to succeed (1993–2000) the renowned founder and Chief of the Liver Department, Prof. Jean-Pierre Benhamou (1927–2008). By the bye, Serge was also Director of the Liver Pathophysiology Research Laboratory of INSERM in the same hospital (1986–1998) and also Secretary General of the European Association for the Study of the Liver (1974–1975).

It would be remiss of me not to mention other captivating facts from his biography. His childhood in Paris was interrupted in the early 1940s by the Nazi German occupation. As a 3-year-old he was separated from his parents (who had previously fled antisemitism in Poland) and his brother, and he was evacuated by the Assistance Publique to a small hamlet, Les Mardelles, near Châtillon-sur-Cher, near the Loire Valley. For 4 years, he was hidden by a compassionate local couple on their small farm in Loir-et-Cher. Erlinger published this poignant period in his young life until his miraculous reunion with his family at war's end, in his graphically documented autobiography *Parcours d'un enfant caché (1941–1945): Une enfance aux Mardelles*.[†††]

After retirement from the University of Paris in 2000, he and his wife relocated to Rognes, a village in Provence, where he augmented his bucolic existence by pursuing studies. in history on the vexed topic of the Israeli-Palestinian conflict ("La représentation du conflit israélo-arabe dans les éditoriaux du *Monde* de 1987 à 2002"), at the university in Aix-en-Provence.

CONFLICT OF INTEREST

Nothing to report.

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[†††] *Journey of a Hidden Child (1941–1945): A Childhood in the Mardelles*. Published by Le Manuscrit, Paris, 2012.

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